PATHOGENIC VARIATION IN PSEUDOMONAS SYRINGAE PV. PHASEOLICOLA STRAINS ON COMMON BEANS

Ariyarathne H.M[1].*,, D. P. Coyne, A.K. Vidaver, K. M. Eskridge, Univ. of Nebraska, Lincoln, NE 68583. [1](Current Address: Dept of Agriculture, RARDC, Bandarawela, Sri Lanka)

Halo blight (HB), caused *by Pseudomonas syringae* pv. *phaseolicola* (Burkh.) (*Psp*), is one of the most important bacterial diseases of common beans (*Phaseolus vulgaris* L.). The HB resistant bean lines PI 150414 (Walker and Patel, Phytopath. 54:952-954,1964) and great northern (GN) Nebraska # 1 sel. 27 (Coyne et al., Pl Dis. Rep. 51:20-24,1967) have been recommended for use in breeding for HB resistance. Breeding for resistance requires knowledge of the pathogenic variation of Psp as well as that of resistant germplasm sources.

Races 1 and 2, of Psp have been identified using the differential reaction of common bean cultivar red mexican 'UI 3' (Walker and Patel, Phytopath. 54:952-954,1964). Race 3 was identified in Africa (Mabagala and Saettler, Pl. Dis. 76:683-686,1992; Taylor et al.,Pl Path. 45 – 469-478,1996). One strain, which does not belong to any of the above three races, was identified in South Africa (Edington, Ann. Rept. BIC. 33:171,1990). Subsequently 9 Psp races were described using 8 differential cultivars (Taylor et al.,Pl. Path. 45:469-478,1996). Also, another race, KM 4, was described using reactions on pods (Szarka, Ann. Rept. BIC. 29:60-61,1986). Fourie (Pl. Dis. 82:307-310, 1995) detected 7 races of HB in South Africa, with Race 8 predominating. The objective of this research was to investigate further the pathogenic variation of Psp strains with emphasis on those collected in Nebraska.

Materials and Methods

Two experiments were planted using 8 differential cultivars\lines and a resistant check line GN Nebr. #1 sel. 27 in a greenhouse, University of Nebraska Lincoln. The 7 following differential cultivars\lines are *P. vulgaris*: 'Tendergreen', ZAA54, ZAA55, ZAA12, red mexican 'UI3', 'Guatemala 196-B', and 'Canadian Wonder'. One *P.acutifolius* (tepary bean) line 1072, differential was used. The cultivar 'Canadian Wonder' is susceptible to all *Psp* strains (Taylor et al ., 1996). GN Nebr. #1 sel. 27 is resistant to races 1 and 2 (Coyne et al.,Pl. Dis. Reptr. 51:20-24, 1967) and race 3 (Taylor et al.,Pl. Path. 45:469-478,1996).

The bean seeds were planted on 10cm clay pots and seedling were thinned to 2 plants per pot 2 weeks after planting. A split-plot design was used with 9 cultivars\lines as a whole-plot treatment in a RCBD. The treatments were replicated twice in 2 growth chambers. Twenty-seven (27) *Psp* bacterial strains were used as split-plot treatments in incomplete blocks with 3 strains applied to each of 3 leaflets of each cultivar in each pot. The random selection of three strains to inoculate 3 leaflets of each cultivar in a pot (incomplete block) was achieved using a different cubic lattice arrangement separately for each cultivar. However, 30 strain treatments including 2 additional strains and a control (inoculation with potassium phosphate buffer (PB) , pH 7.1) were available for evaluation. To include these 3 additional strain treatments for each cultivar, the 3 leaflets from an additional pot were used for inoculation giving a total of 10 pots and 30 strain treatments for each cultivar in each chamber. The *Psp* strains were grown on NBY medium for 72 hours at 25 C. The cultures were gradually transferred to 5 ml of 12.5 mm potassium phosphate buffer (PB) (pH 7.1) until diluted to read 0.1 O>D. on a Bausch and Lomb Spectronic 20 spectrophotometer set at 640 nm. By adding a measured bacterial suspension to PB, final concentration of 1 x 10^6 colony-forming units (cfu)/ml were prepared and used for inoculations. About 20 day old seedlings with 3/4 expanded first trifoliolate leaves were used for

inoculations. The lower sides of the first trifoliolate leaves were inoculated, using water-soaking inoculation method (Schuster, Phytopath. 45:519-520, 1955). Then plants were kept in the growth chambers under 20° ± 2 C and 14 hour dark period. The mean light intensity of the growth chambers at the plant canopy was 126 ± 22 umol sec-1 m -2. Leaf disease reactions were recorded 14 days and 21 days after inoculation. Disease symptoms of leaves with systemic chlorosis were scored, using 1-5 scale, where an incompatible reaction (-) was designated as ratings 1 and 2, while a compatible reaction (+) was designated with ratings of 3 or greater. The data were initially analyzed using the combined intra-inter block analysis with SAS Mixed procedure. Several contrasts were also tested in order to understand possible cultivars x strain interactions.

Results and Discussion

The results showed no effects from the incomplete blocks (pots) term in the model ($P = 0.5669$). Therefore, the data including observations of all 29 strains were re-analyzed as a standard split-plot design using the SAS GLM procedure considering growth chambers (replicates) as random effects. Disease reactions due to cultivars, strains, and cultivar x strain interaction were significantly different ($P < 0.0001$) indicating different virulence of *Psp* strains. The cultivar x strain interaction was expected because of the use of a known set of bean differentials. *Psp* strains collected in Nebraska reacted similarly to other strains tested except on line ZAA 12 where a significant interaction was found ($P = 0.0245$). A cultivar x strain interaction was not observed between strains from the USA and those from other countries ($P = 0.2399$). Twenty-four Psp strains collected in the USA were more pathogenic than the other strains on all of the cultivars ($P = 0.0001$).

The findings of combined intra-inter block analysis here suggested that if many strains need to be tested then 3 leaflets of the first trifoliolate leaves can be inoculated with 3 different strains. Even though this involves an incomplete block to test the strains, the experiment can be analyzed as a RCBD. Strains 1990 Beryl and 1370A NCPPB did not fluoresce under UV light. Some *Psp* strains do not fluoresce but have other characteristics of *Psp* including pathogenicity on common bean (Taylor et al., Pl. Path. 45:469-478, 1996). Color of the above two *Psp* strains were differed from other strains on King`s B medium.

Canadian Wonder was susceptible to all the bacterial strains tested and expressed typical HB symptoms. The tepary line 1072 expressed systemic chlorosis with the majority of the compatible *Psp* strains. GN Nebr. #1 sel. 27 (check) was resistant to all the *Psp* strains and Coyne et al. (Ann. Reptr. BIC. 27:161,1984) found that it was resistant to four *Psp* strains.

Based on the reaction on the eight differential cultivars established by Taylor et al. (Pl. Path. 469-478,1966). the 29 *Psp* strains were placed into 5 groups. Three groups were similar to races 1, 6, and 7 while 2 groups were different from any of the 9 races described by Taylor et al. (Pl. Path. 469-478,1966). A group of 4 strains was designated as a new race 10. Two strains, strain 1990 Beryl (NE) and 1370A NCPPB, were designated as a new race 11. Five races, 1, 6, 7, 10 , and 11 were identified among the 15 NE strains with 1, 9, 1, 3 and 1 strains matching for above races, respectively. Sixteen strains (55%) were classified as race 6 and were compatible on all 8 differential cultivars\lines. Race 6 was the most frequently observed race by Taylor et al. (Pl. Path. 45:469-478,1996) with 32% of 172 *Psp* strains collected from several countries belonging to race 6. The information obtained in this study will be useful to breeders in determining sources of resistance to use against races\strains particularly prevalent in the western high plains of the US.